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Bilthoven

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Synthesis of deuterated anabolic compounds

G.Zomer

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Abstract and conclusions

This report describes the synthesis of the deuterated anabolic compounds* diethylstilbestrol-D_6, hexestrol-D_4, dienestrol-D_2, methyltestosterone-D_3, zeranol-D_4, trenbolone-D_2, nortestosterone-D_2, and medroxyprogesterone-D_3. The synthetic routes that were used are flexible i.e. it is possible to introduce more deuterium atoms into the anabolic compounds.

* A list of trivial compound names together with chemical names according to "Chemical Abstracts" can be found at Appendix 2.

1. General Introduction

Gas chromatography - mass spectrometry (gc-ms) is considered to be the most reliable technique to analyse very small amounts of chemical compounds which are present in complicated biological matrices. By using internal standards, it is possible with gc-ms to quantify these compounds. The ideal internal standard is that compound which only differs in mass from the compound to be analysed. In this study which was concerned with the detection of anabolic compounds in urine of cattle (for a review see McLachlan, 1980), deuterated analogues were chosen. Deuterium atoms can be incorporated into the compounds of interest with relatively simple and cheap methods. Isotopes of carbon (^{13}C), or oxygen (^{18}O) cannot normally be incorporated in a simple way. Moreover, these isotopes are more expensive. On the other hand, there are advantages in using ^{13}C -and to a lesser extend ^{18}O. These isotopes generally cannot be exchanged as readily as deuterium atoms (this depends of course on the position of the isotope). That is why in every case these factors of exchangeability, ease of introduction, and cost have to be considered. In general it can be stated that no isotope should be used which can be exchanged measurably under the working conditions of the test for which the internal standard is developed.

A potential disadvantage of deuterium is that through the use of a large number of deuterium atoms in a compound, physical properties like chromatographic behaviour change more than when other elements are used. This effect restricts the amount of deuterium atoms that can be introduced. From an analytical point of view it is better to introduce more isotopic atoms into the compound to lower the mass spectrometrical background. In practice, the synthetic possibilities, together with what is theoretically needed wil determine the structure of the internal standard.

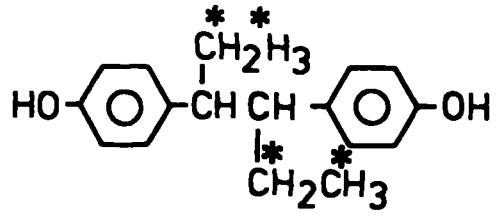
2. Deuterated stilbene anabolics

2.1. Introduction

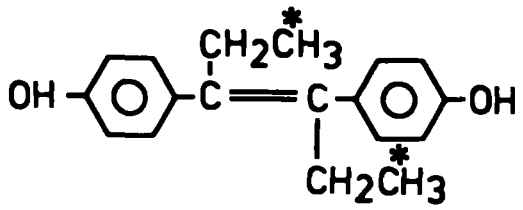
The syntheses of diethylstilbestrol-D_3* (DES-D_3) and diethylstilbestrol-D_5 (DES-D_5) have been described (Marshall et al, 1975; Metzler, 1978 respectivily). The routes that were followed by these authors are collected in appendix 1. The drawback of these synthetic routes is that they cannot be used to prepare other stilbene anabolics. Another disadvantage is that the two deuterium atoms at the allylic methylene position in DES-D_5 are easily exchanged with hydrogen (Liehr and Ballatore, 1982). The synthesis of DES-D_4 recently has been described (Stein and v.d.Willigen, 1982). This compound cannot effectively be used as an internal standard because of possible deuterium-hydrogen exchange which might take place during the analysis.

* See Appendix 2 for a list of trivial and chemical compound names (according to "Chemical Abstracts")

The positions which ideally should be used to incorporate deuterium into stilbene anabolics are denoted in figure 1. Because of the not very well accessible aromatic positions (however, see Liehr and Ballatore, 1982), the aliphatic (HEX and DES) and vinylic (DE) positions were considered as being the best candidates for the introduction of deuterium. Furthermore, symmetry considerations played a role in the design of the synthetic schemes.



Hexestrol



Diethyl stilbestrol

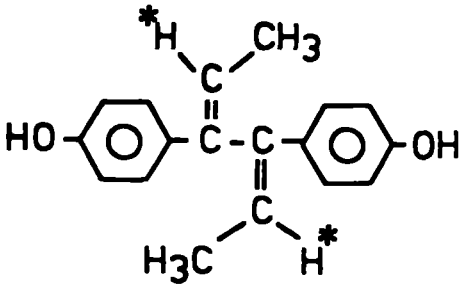
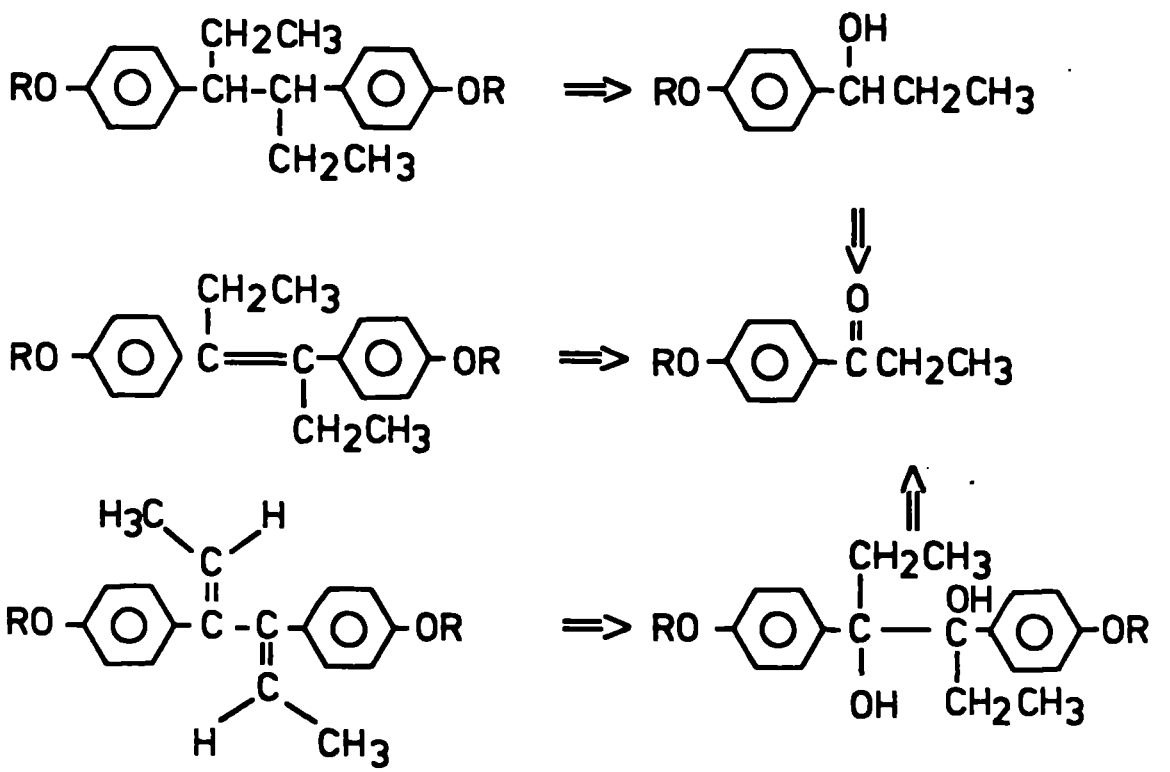


Figure 1

2.2. Synthetic plan

2.2.1. Retro synthesis of deuterated stilbene anabolics

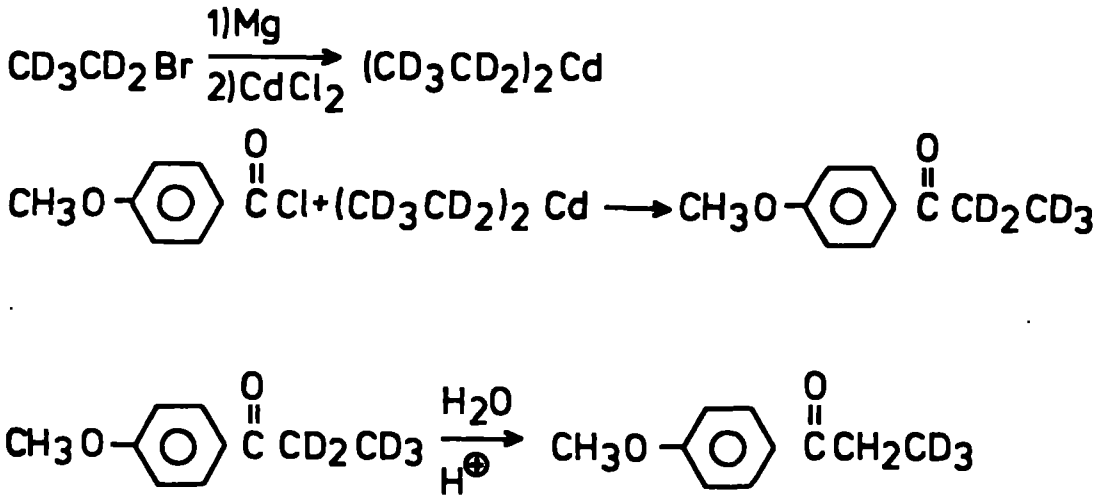
The retro synthetic scheme, starting from DES, HEX, and DE is shown in scheme 1. As can be seen from scheme 1, it is possible- at least on paper- to prepare DES, HEX, and DE from the same precursor, p-hydroxypropiophenone. So, the next problem will be to introduce deuterium atoms into p-hydroxypropiophenone.



Scheme 1

2.2.2. Deuterated p-hydroxypropiophenone

p-Hydroxypropiophenone, being an aromatic ketone easily can exchange its α hydrogen atoms. This exchange can be performed with acid or base catalysis. In this study the exchange was performed using acid catalysis (p-toluenesulphonic acid).

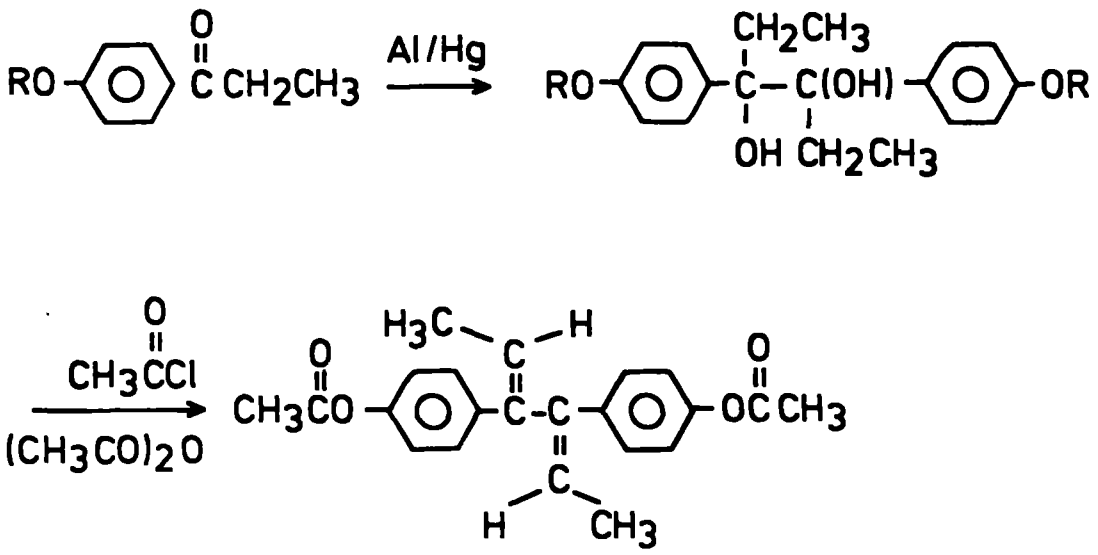


Scheme 2

To deuterate p-hydroxypropiophenone at the methyl position needed for the synthesis of DES-D_6 the following route was designed (Scheme 2). In this route pentadeuterated ethylbromide was converted to perdeuterated diethylcadmium via the Grignard reagent. This organometallic compound could react with acyl chlorides like p-anisoylchloride to form ketones like p-methoxypropiophenone. The thus prepared p-methoxypropiophenone contained five deuterium atoms per molecule. Two of these deuterium atoms at the α positions could be back exchanged with hydrogen using acid or base. Under these conditions the methyl deuterium atoms were not exchanged.

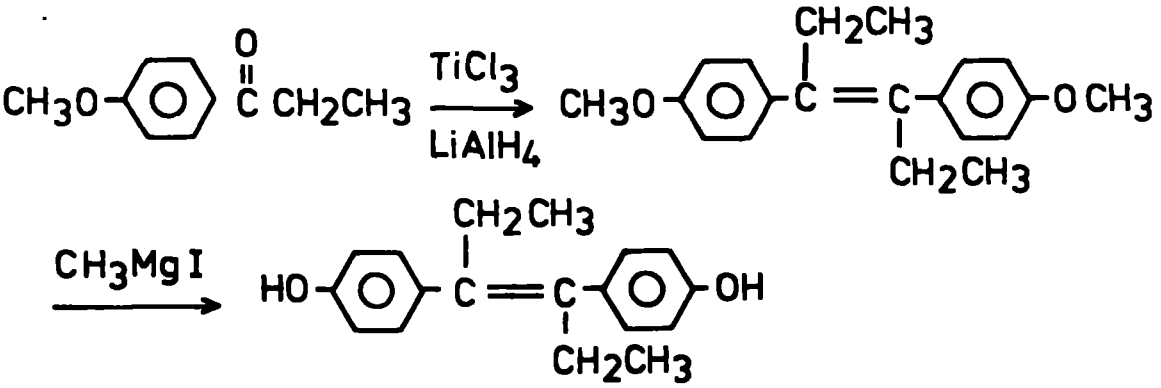
2.2.3. Coupling reactions

The (protected) p-hydroxypropiophenone could be coupled reductively in two ways: The so-called pinacol coupling gave a diol (pinacol) as shown in scheme 3.



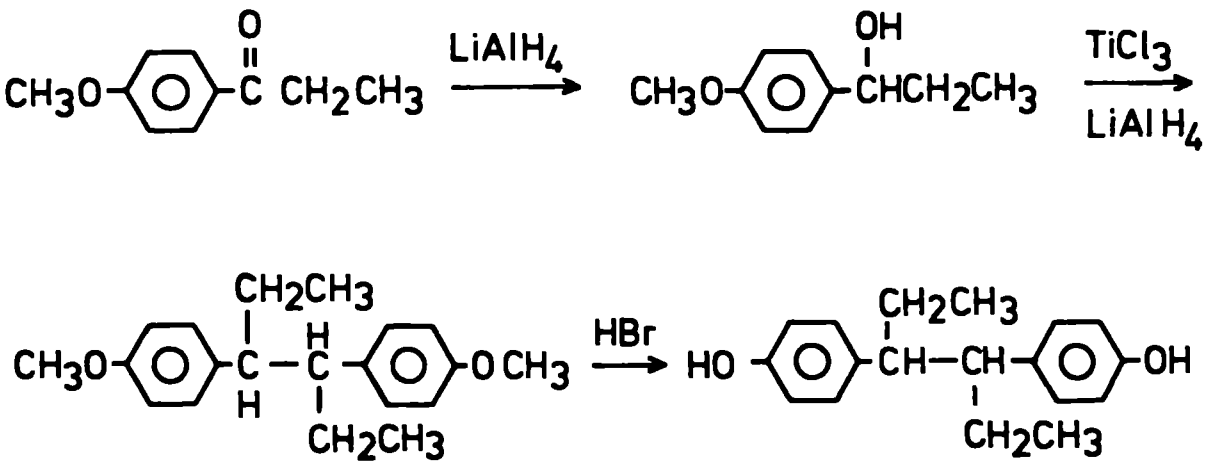
Scheme 3

Dehydration of the pinacol using acetyl chloride and acetic anhydride gave dienestrol acetate. Hydrolysis of the acetate functions yielded dienestrol.



Scheme 4

The second way to couple ketones is to react the ketone with titaniumtrichloride and lithium aluminiumhydride in tetrahydrofuran (THF) (scheme 4) (McMurry). This reaction gave olefines directly. Thus, when this reaction was applied to p-methoxypropiophenone the dimethyl ether of DES was produced. The methyl groups could be split off by the action of methyl magnesiumiodide to give DES. The same reaction, when applied to alcohols gave symmetrical aliphatic compounds. Thus, hexestrol could be prepared from p-methoxypropiophenone by first reducing the ketone function with lithium aluminiumhydride, and then applying the coupling reaction on the alcohol. Hexestrol dimethylether could be converted to hexestrol by hydrogen bromide (Scheme 5).



Scheme 5

2.3. Results (Zomer, d.d. 1983, 1984)

2.3.1. The synthesis of dienestrol-D_2

2.3.1.1. The synthesis of p-hydroxypropiophenone-D_2

p-Hydroxypropiophenone (5 g) in a mixture of dioxane / D_2O (1/1, 100 ml) was heated under reflux in a nitrogen atmosphere with p-toluenesulphonylchloride (1 g) overnight. After cooling to room temperature the mixture was saturated with salt and the aqueous layer separated and extracted with diethyl ether. The organic layers were combined, dried and evaporated to give 4.8 g (95%) of deuterated p-hydroxypropiophenone (NMR ($CDCl_3$): 1.2 (s,3H), 6.8 and 7.8 ppm (AB, 4H). p-Methoxy-propiophenone-D_2 was prepared using the same conditions as described above. NMR ($CDCl_3$): 1.2 (5,3H) 3.8 5,3H 6.9 and 7.9 ppm (AB, 4H).

2.3.1.2. The synthesis of dienestrol-D_2

Aluminium foil (4.5 g) was treated with a 2% $HgCl_2$ solution (150 ml). After 3 minutes the amalgan was washed with water, absolute ethanol, and diethyl ether. The dry amalgan was covered with dry THF (ca. 100 ml). To this mixture p-hydroxypropiophenone-D_2 (2.5 g) was added. After one hour, the mixture was gently heated under reflux in a nitrogen atmosphere for 3 hours. The reaction mixture was filtered, dried, and evaporated to afford 2 grams of the crude pinacol. This product, dissolved in 10% NaOH (20 ml) was treated with acetic anhydride (10 ml). The diacetylated product consisted of a mixture of dl- and meso pinacol. This mixture was heated under reflux with a mixture of acetic anhydride/ acetyl chloride (2/1, 100 ml) for 2 hours. The mixture was poured into water, and neutralised with powdered potassium carbonate. Extraction with CH_2Cl_2, drying, and evaporation of

the solvent yielded crude dienestrol-D_2 acetate. Deacetylation was achieved by treatment of the product with 10% NaOH in ethanol. The product was purified by flash chromatography (SiO_2, hexane/ethylacetate 2/1), followed by recrystallization from benzene 0.6g (28%). The product thus obtained had a purity >95% as determined with NMR, HPLC, and GLC. NMR (CD_3COCD_3): 1.4 (s,6H), 6.7-7.1 (AB, 8H), 8.0 ppm (s,2H). MS m/e (%): 268 (100), 253 (55), 238 (60), 146 (35), 122 (52).

2.3.2. The synthesis of [1,1,1,6,6,6-D_6]-diethylstilbestrol (DES-D_6)

2.3.2.1. The synthesis of [1,1,1-D_3]-p-methoxypropiophenone

Perdeuterated diethylcadmium was prepared from ethylbromide-D_5 (8 g), magnesium (1.8 g), and cadmium(II)chloride (6.8 g) in benzene (50 ml). To this solution was added with stirring p-anisoylchloride (11.9 g) dissolved in benzene (40 ml). During the addition the temperature rose to 45 °C. After stirring the reaction mixture for 2 hours at 45 °C it was poured onto ice and acidified with concentrated sulfuric acid. The resulting mixture was left overnight. Extraction with diethyl ether, washing of the organic layers with water, drying, and evaporation gave deuterated p-methoxypropiophenone. The crude product was refluxed for 1 hour in dioxane, containing 20ml of 1N HCl. Work-up as described above yielded crude [1,1,1-D_3]-p-methoxypropiophenone. It was purified by distillation to afford 5.7 g (50%) of pure [1,1,1-D_3]-p-methoxypropiophenone (b.p. 138-140 °C / 14 mm) as a colourless oil that solidified completely after one night at 6 °C. NMR ($CDCl_3$): 2.6 (bs,2H), 3.5 (s,3H), 6.7-7.8 ppm (AB,4H).

2.3.2.2. The synthesis of DES-D_6

$TiCl_3$ (10 g) in dry tetrahydrofuran (THF, 60 ml) was stirred during 15 minutes. $LiAlH_4$ (1.25 g) was added carefully in small portions under a nitrogen atmosphere. A vigorous reaction occurred (gas evolution, generation of heat) depositing a black precipitate. To complete the reaction the mixture was refluxed for 30 minutes. After cooling to room temperature a solution of [1,1,1-D_3]-p-methoxypropiophenone (5.7 g) in 20 ml of dry THF was added in 10 minutes. The resulting mixture was refluxed for 4 hours under nitrogen. The cooled reaction mixture was diluted with water (100 ml), and extracted with dichloromethane. The organic layers were washed with water, dried, and evaporated to give an oil. This oil was adsorbed on silica and eluted with petroleum ether (bp 40-60 °C) / ethyl

acetate (20:1) to yield dimethyl DES-D_6 (2.2 g, 50%) as a mixture of cis- and trans isomers. NMR ($CDCl_3$): 2.4 (bs,2H, trans), 2.7 (bs,2H, cis), 3.8 (s,3H), 6.7-7.2 ppm (AB,4H).

Methyl magnesiumiodide (150 mmol) was prepared in diethyl ether. Dimethyl DES-D_6 (2.2 g) was dissolved in this solution. The diethyl ether was removed by distillation, and the resulting mixture heated for 1 hour at 160-180 °C under nitrogen. After cooling diethyl ether (100 ml) was added followed by the careful addition of water (10 ml). The mixture was acidified with 10% sulfuric acid, and stirred until both layers were clear. The aqueous layer was extracted with diethyl ether, the organic layers were dried, and evaporated. There was obtained 1.8 g (90%) of a mixture of cis- and trans DES-D_6 (cis: trans 4 : 1). Isomerization was accomplished by refluxing the crude product in chloroform (100 ml) until NMR indicated the presence of a cis / trans ratio of 1 / 3. Pure [1,1,1,6,6,6-D_6]-E-3,4-di(4-hydroxyphenyl)-hex-3-ene (trans DES-d6) was obtained by crystallisation of the crude product from benzene (mp 170-2 °C). The purity of the product was judged to be >95% by NMR, HPLC, and GC. NMR(CD_3COCD_3): 2.1 (bs,4H), 6.7-7.1 (AB,8H), 8.3 ppm (s,2H).

2.3.3. The synthesis of [2,2,5,5-D_4]-hexestrol (HEX-D_4)

2.3.3.1. The synthesis of [2,2-D_2]-1-propanol,1-(4-methoxyphenyl)

[2,2-D_2]-p-Methoxypropiophenone (1.64 g) in 10 ml of absolute THF was added to $LiAlH_4$ (0.4 g) in 20 ml of THF. After stirring for 15 minutes the mixture was cautiously treated with excess water. Filtration and removal of the solvent by distillation afforded [2,2-D_2]-1-propanol,1-(4-methoxyphenyl) (1.5g, 90%).

NMR ($CDCl_3$): 0.8 (bs,3H), 3.7 (s,3H), 6.8-7.3 ppm (AB,4H).

2.3.3.2. The synthesis of hexestrol-D_4

To a stirred slurry of $TiCl_3$ (3 g) in 60 ml of THF was added $LiAlH_4$ (0.4 g), followed by 15 ml of THF. After stirring for 30 minutes [2,2-D_2]-1-propanol-1(4-methoxyphenyl) (1.04 g) in THF (10 ml) was added over 5 minutes. The mixture was stirred and heated under reflux in a nitrogen atmosphere for 2 hours. After cooling to room temperature the mixture was poured into water and extracted with dichloromethane (3 x 40 ml). The organic layers were washed with water, dried, and evaporated to yield deuterated hexestrol dimethyl ether as a mixture of meso- and dl isomers. The meso isomer crystallised from 15 ml of methanol (yield 0.4 g, 40%).

NMR ($CDCl_3$): 0.5 (s,6H), 2.47 (s,2H), 3.8 (s,6H), 6.7-7.3 ppm (AB, 8H).

A solution of the dimethyl ether (0.4 g) in 8 ml of HBr in acetic acid (40%) was heated under reflux for 3 hours. The reaction mixture was cooled to room temperature, poured onto ice, and extracted with diethyl ether (3 x 50 ml). The combined organic layers were washed with water, and with 5% $NaHCO_3$ solution. The ether solution was extracted with 5% NaOH, and the aqueous extracts washed with diethyl ether. The aqueous layer was acidified with diluted sulfuric acid, and then extracted with diethyl ether. The combined extracts were washed with water, dried, and evaporated yielding meso hexestrol-D_4. Recrystallization from aqueous ethanol afforded 250 mg (60%) of white needles (mp 183-5 °C).

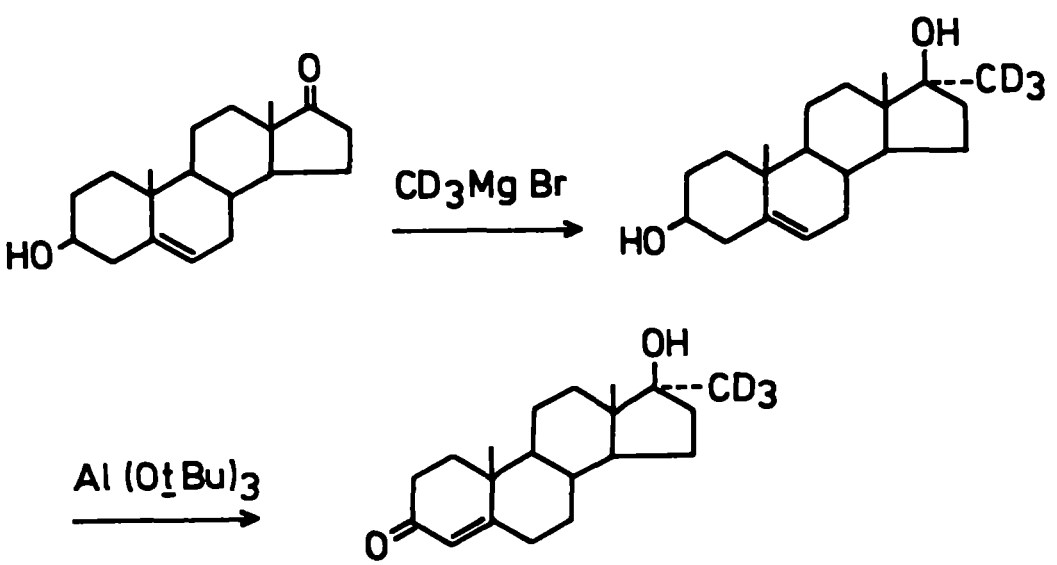
NMR (CD_3COCD_3): 0.49 (s,6H), 2.4 (s,2H), 5.0 (bs,2H), 6.6-7.1 ppm (AB,8H). MS m/e (%): 137 (100).

3. The synthesis of deuterated steroid anabolic compounds

3.1. The synthesis of 17-alpha-methyltestosterone-D_3

3.1.1. Synthetic route

The most obvious position to incorporate deuterium atoms into 17-α-methyltestosterone (MT), is at the 17 α methyl group (i.e. in stead of a normal methyl group, a deuterated methyl group has to be incorporated in the synthetic route). The synthetic route that was followed is depicted in scheme 6. Essentially, this is the classical way to prepare MT. The only difference being that in stead of methyl magnesiumiodide [1,1,1-D_3]-methyl magnesiumiodide was used in the present study.



Scheme 6

3.1.2. Results

[1,1,1-D_3]-Methylmagnesiumbromide was prepared from 10 ml of [1,1,1-D_3] methylbromide and 4.9 g of magnesium in THF. The solution containing the grignard reagent was cooled in an icebath, and treated with a solution of dehydroisoandrosterone (10 g) in THF (60 ml). The cooling bath was removed, and the reaction mixture gently heated under reflux for 16 hours. After cooling to room temperature, the reaction was worked up by pouring it into icewater, acidification with diluted sulfuric acid, and extraction with chloroform afforded the methylated compound (10.2 g, 90%) which could be purified by recrystallization from ethyl acetate.

NMR ($CDCl_3$): 0.85 (s,3H), 1.05 (s,3H), 3.5 (bs,1H), 5.4 ppm (bs,1H).

The diol (1.4 g), dissolved in a mixture of acetone (30 ml), and benzene (20 ml) was treated with aluminium tri-tert-butoxide (2.75 g) in benzene (100 ml). The mixture was refluxed for 20 hours under nitrogen. The light yellow coloured reaction mixture was cooled to room temperature, washed with 10% H_2SO_4 (4 x 25 ml), water, dried, and evaporated to afford the crude 17-α[D_3]-methyl testosterone. Recrystallisation from diethyl ether gave the pure compound (0.6 g, 45% mp 162-4 °C).

NMR ($CDCl_3$): 0.88 (s,3H), 1.17 (s,3H), 5.73 ppm (bs,1H).

3.2. The synthesis of trenbolone-D_2

3.2.1. Synthetic route

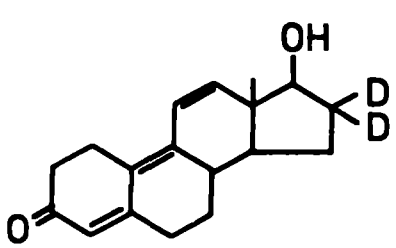
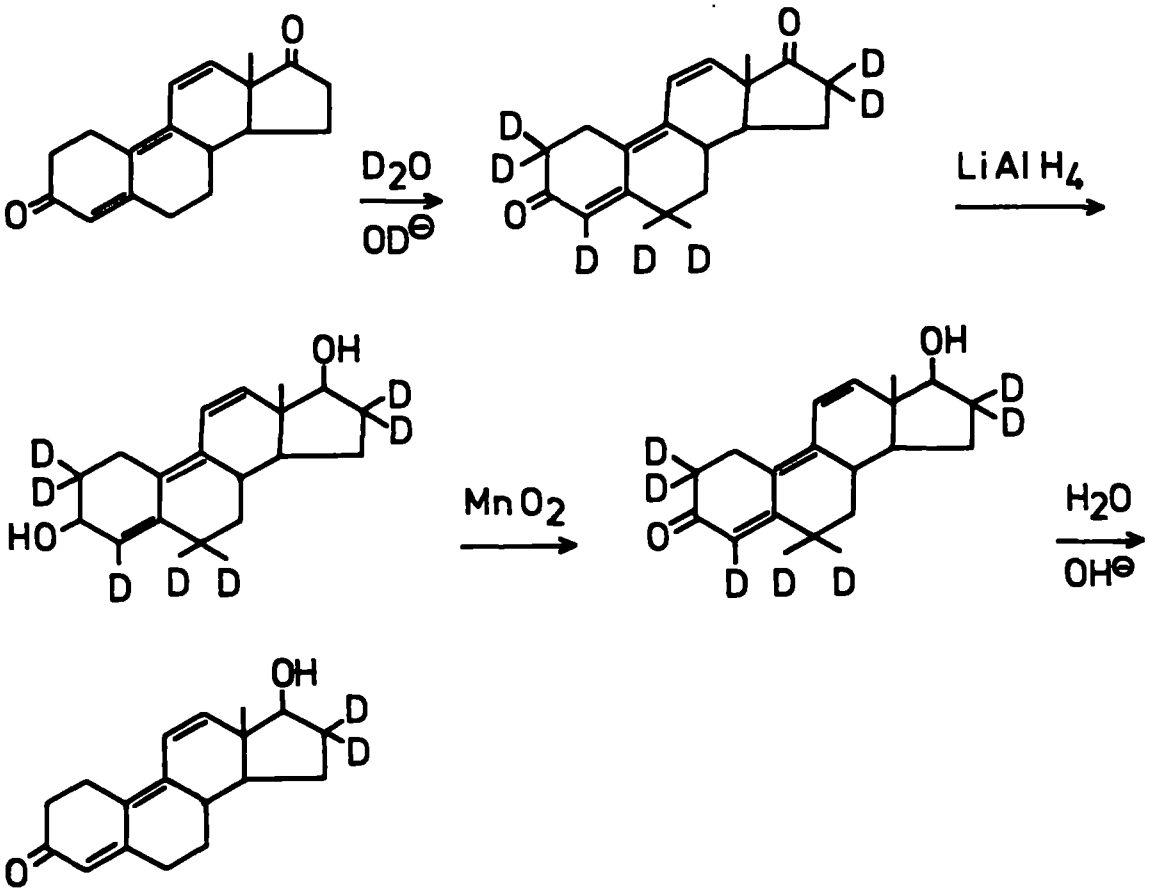


Figure 2

The most straightforward positions to incorporate deuterium atoms into the trenbolone molecule are denoted in figure 2. These positions are easily accesible by the following route: Oxidation of the 17-OH function gives the 17-ketone (trendione). In trendione the positions 2, 4, 6, and 16 can be exchanged with deuterium. Reduction of the two ketone moieties will produce a mixture of isomeric diols. Allylic oxidation of the 3-OH function will restore the 3-keto function without oxidation of the 17-OH group. Finally, back exchange with water will give trenbolone-D_2 (Scheme 7).



Scheme 7

3.2.2. Results

Trendione (100 mg), D_2O (10 ml), and sodium carbonate (200 mg) in dioxane (10 ml) was refluxed for 2 hours. The solvents were removed by distillation, and the procedure repeated with fresh D_2O. The crude product was dissolved in ethanol (2 ml). $NaBH_4$ (100 mg) in ethanol (10 ml), and D_2O (2 ml) was added. After 15 minutes at room temperature, the reaction was

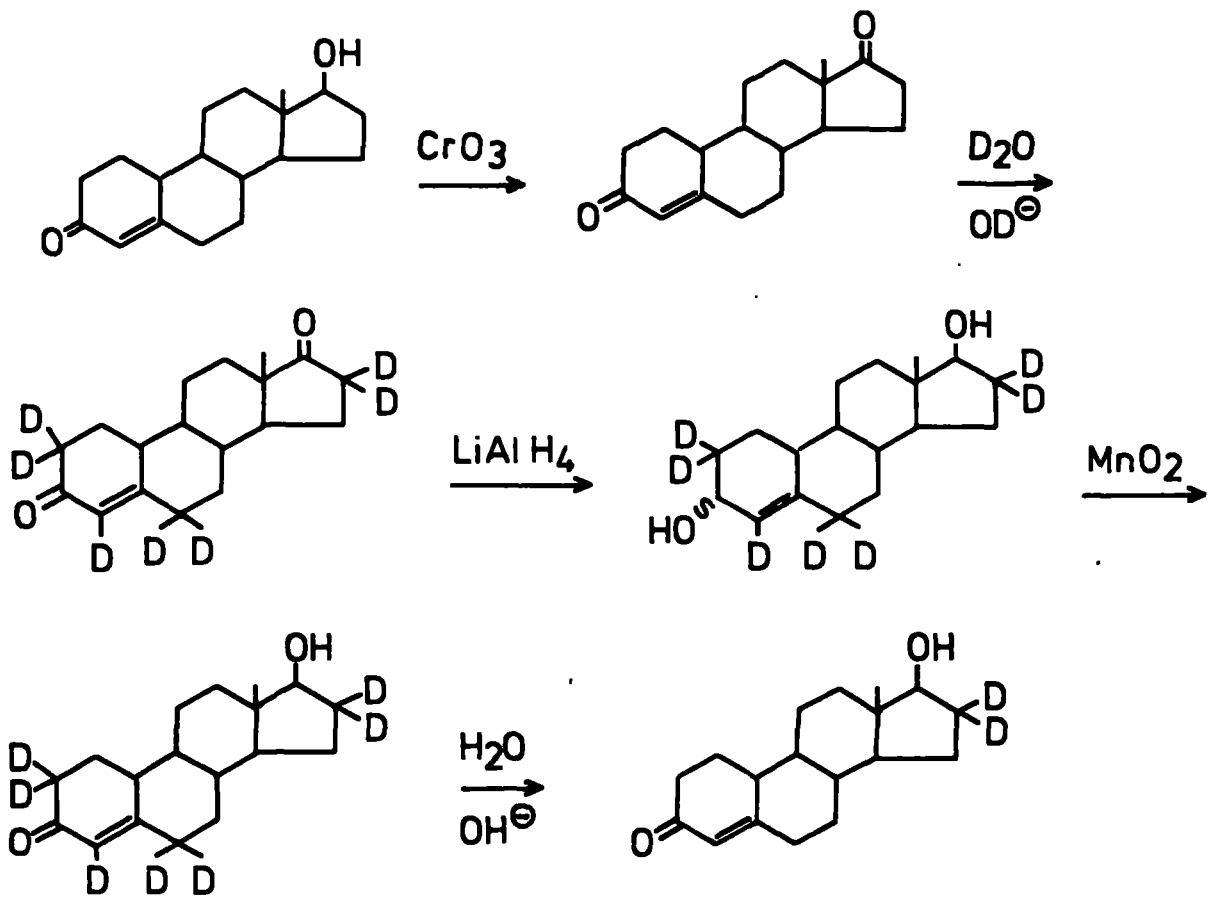
quenched with 1N HCl. The products, two isomeric alcohols were extracted with diethyl ether, the organic layers were washed with water, dried, and evaporated to afford the diols. The crude product was oxidized with activated MnO_2 (0.9 g) in chloroform (10 ml). After 2 hours the reaction mixture was filtrated, and the solvent removed to give crude trenbolone-d2. The product was purified by flash chromatography (SiO_2, light petroleum/ethyl acetate 1/1), followed by crystallization from ethyl acetate/light petroleum (0.025 g, 25%).

NMR ($CDCl_3$): 0.9 (s,3H), 2.9 (bs, 1H), 5.7 (bs,1H), 6.5 ppm (s,2H).

3.3. The synthesis of 19-nortestosterone-D_2 (NT-D_2)

3.3.1. Synthetic route

The synthetic route to prepare NT-D_2 involves the same steps as described for trenbolone-D_2 (i.e. oxidation, deuterium exchange, reduction, selective oxidation, and back exchange (Scheme 8)).



Scheme 8

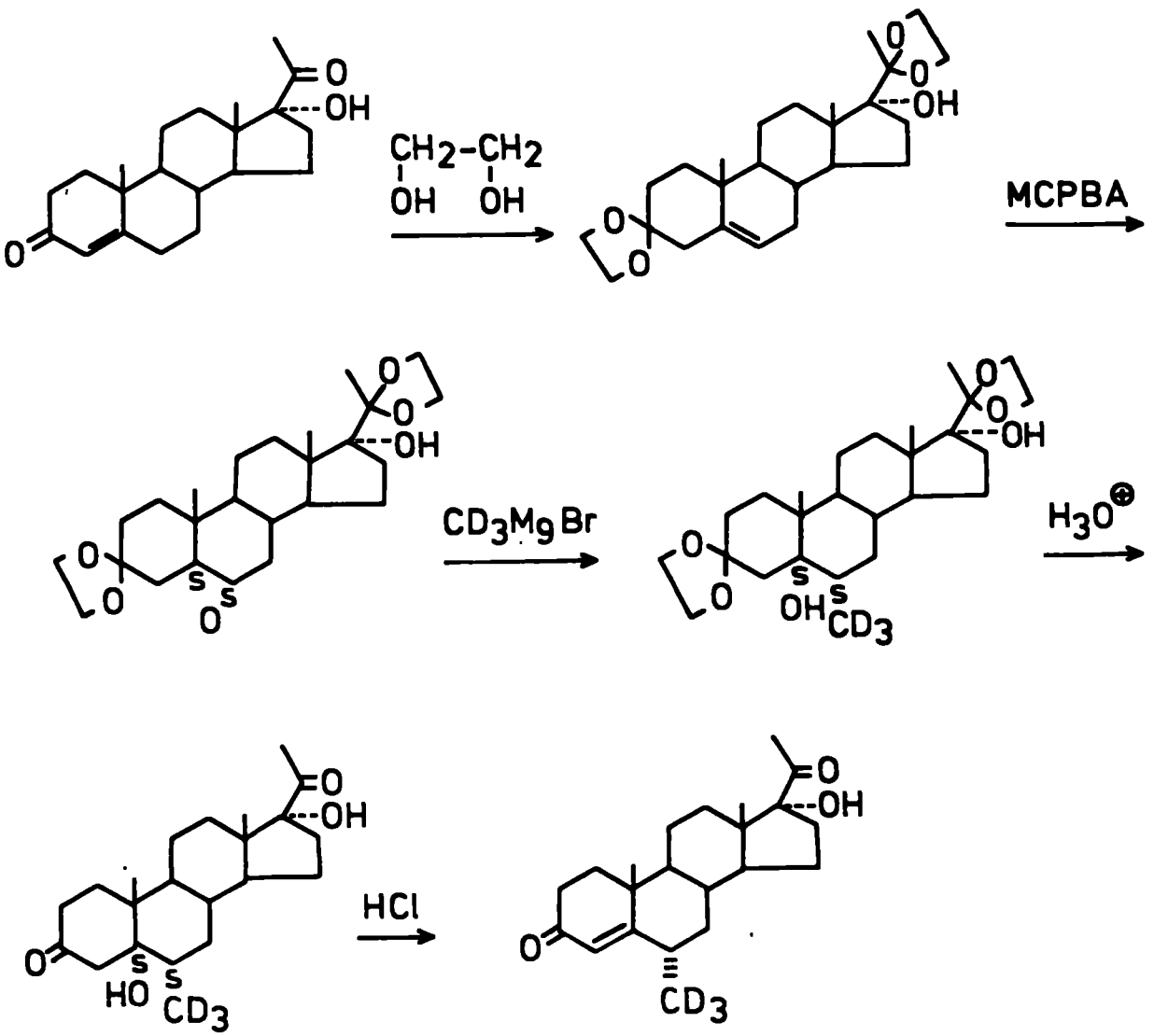
3.3.2. Results

19-Nortestosterone (1 g) dissolved in acetone (50 ml), was oxidized with 3 equivalents of Jones' reagent at 0 °C. After 30 minutes, the excess of Jones' reagent was reacted with 2-propanol. Water (20 ml) and chloroform (100 ml) was added. The organic layer was washed with water (3 x 50 ml), dried, and evaporated to give the crude diketone. This product was refluxed in a mixture of D_2O (20 ml) and acetonitril (30 ml) containing sodium carbonate (2 g). This mixture was refluxed for 3 hours. The solvents were removed by distillation under reduced pressure, and the exchange reaction repeated with fresh D_2O (20 ml), and acetonitril (30 ml). The solvents were evaporated, and the residue dissolved in CH_2Cl_2. After drying the solvent was evaporated. The residue was dissolved in THF (20 ml), and treated with excess $LiAlH_4$. The mixture was refluxed for 30 minutes. Cautious addition of D_2O followed by filtration and evaporation of the solvent gave two isomeric diols. These diols were dissolved in chloroform (100 ml), and treated with activated MnO_2 (13 g). After 3 hours at room temperature, the mixture was filtrated, and evaporated. The crude product was treated with a mixture of sodium carbonate (1 g) in water (100 ml), and dioxane (50 ml) at reflux temperature. The product was extracted with ether, the ether layers were washed with water, dried, and evaporated to afford 19-nortestosterone-D_2. It was purified by flash chromatography (SiO_2, Ethyl acetate / petroleum ether, 1 / 3), to give the pure compound (0.03 g, 5%).

NMR($CDCl_3$): 0.8 (s,3H), 3.6 (bs,1H), 5.7 ppm (bs,1H).

3.4. The synthesis of medroxyprogesterone-D_3

3.4.1. Synthetic route



Scheme 9

The synthetic route (Babcock, 1958) that was followed to prepare medroxyprogesterone-D_3 (MP-D_3) is shown in scheme 9. The starting material is 17-hydroxyprogesterone. The two ketone functions were protected as the ketals. During the ketalization the 4,5 double bond shifts to the 5,6

position. Oxidation of this double bond using m-chloroperbenzoic acid afforded two isomeric epoxides. Reaction of these epoxides with deuterated methyl magnesiumbromide gave a number of isomeric diols. Deketalization with hydrochloric acid was accompanied by isomerization and gave medroxyprogesterone-D_3.

3.4.2. Results

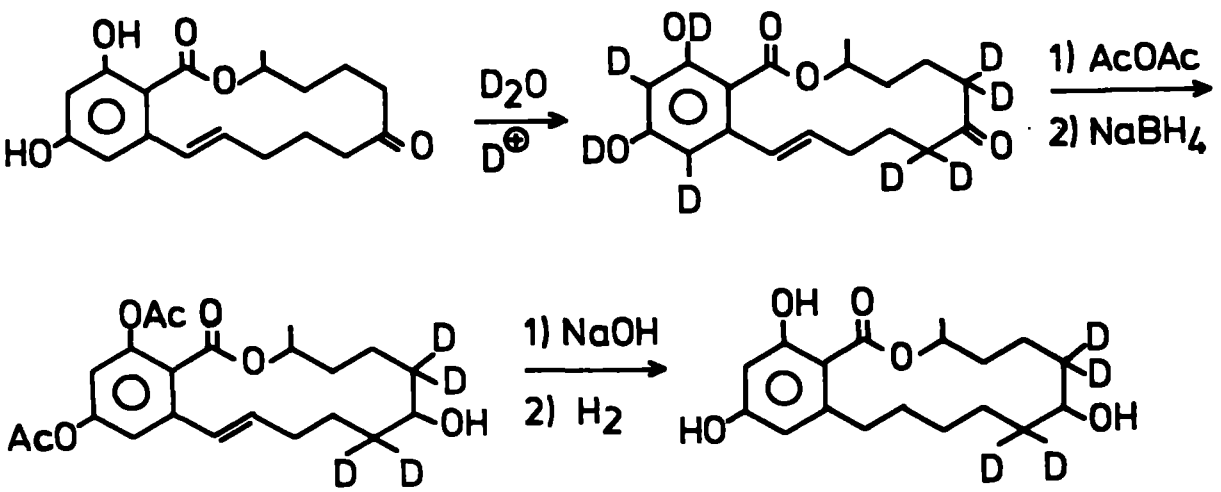
17-Hydroxyprogesterone (10 g) in toluene (500 ml) was refluxed with ethylene glycol (25 ml) and pyridinium p-toluenesulfonate (PPTS, 2g) using a water separator. After 4 hours the reaction was complete. The solvent was removed by distillation under reduced pressure, and the residue dissolved in a mixture of ethyl acetate and water. The organic phase was separated, washed with water, dried, and evaporated. The crude bis-ketal was recrystallized from acetone / petroleum ether to give 8.5 g of pure compound. The bis-ketal (2.5 g) in CH_2Cl_2 (100 ml) was cooled in an ice bath, and treated with m-chloroperbenzoic acid (1.25 g). The mixture was placed in a refrigerator with occasional shaking. After 24 hours the mixture was shaken with excess 5% NaOH. The organic layer was separated, washed with water, dried, and evaporated to give the crude product, which could be recrystallized from acetone. The mixture of epoxides in THF (100 ml) was added to a cooled solution of [1,1,1-D_3]-methyl magnesiumbromide in THF. The cooling bath was removed, and the mixture gently refluxed for 10 hours. After cooling to room temperature overnight, the mixture was poured onto ice. Acidification with 10% sulfuric acid, washing of the organic phase with water, and evaporation gave the methylated products. Deketalization was performed by refluxing the crude products in 80% aqueous acetone with PPTS (1 g) for 2 hours. Water (50 ml) was added, and the acetone removed. The white precipitate which formed was collected by filtration. Chloroform (100 ml) was saturated with hydrochloric acid. To the resulting solution the crude hydroxy-ketone was added with stirring. Stirring was continued until tlc indicated that the reaction was complete. The solvent was removed, and the residue purified by flash chromatography (SiO_2, Petroleum ether / ethyl acetate 3 / 1).

NMR ($CDCl_3$): 0.7 (s,3H), 1.2 (s,3H), 2.3 (s,3H), 5.7 (d,1H).

4. The synthesis of deuterated zeranol (Z-D_4)

4.1. Synthetic route

To synthesize deuterated zeranol, the enolisable positions of zearalanone, the precursor of zeranol seems to be the most obvious to introduce deuterium atoms. As a result, the synthetic route looks as shown in scheme 10. Using acid catalysis, the four enolisable positions of zearalenone were readily exchanged with D_2O. Unexpectedly, under the reaction conditions, also the aromatic positions became deuterated. After acetylation of the phenolic OH groups, the reduction of the 6-ketone function was accomplished with sodium borohydride. Deacetylation, followed by hydrogenation of the double bond gave zeranol-D_4.



Scheme 10

4.2. Results

Zearalenone (0.7 g) dissolved in dioxane (20 ml) was added to D_2O (30 ml). After the addition of p-toluenesulfonylchloride (25 mg), the mixture was refluxed under nitrogen for 90 hours. After cooling to room temperature, the mixture was extracted with diethyl ether. The ether layers were dried, and evaporated. The exchange reaction was repeated using 30 ml of D_2O, 30 ml of dioxane, and 25 mg of p-toluenesulfonylchloride. After refluxing for 45 hours the reaction mixture was worked-up as above to give a white foam. This was dissolved in a mixture of acetic anhydride / pyridine (25 ml 3 / 2). After two hours at room temperature the solvents were removed by distillation under reduced pressure. The resulting diacetate was dissolved in absolute ethanol (50 ml). A solution of $NaBH_4$ (250 mg) in ethanol (30 ml) was added. After stirring for 1 hour at room temperature, the mixture was acidified with 10% sulfuric acid. Extraction with CH_2Cl_2, washing of the extracts with water, drying, and evaporation of the solvent gave a white foam. This product was stirred for one night with three NaOH pellets in a mixture of water (20 ml) and ethanol (20 ml). Ice was added. The mixture was acidified with 10% sulfuric acid. Extraction with diethyl ether, washing of the ether layers with 5% $NaHCO_3$ solution, and with brine, drying, and evaporation gave a white foam (350 mg). According to NMR spectroscopy the foam consisted of two isomeric olefines. This mixture was dissolved in ethanol (30 ml), and after the addition of cyclohexene (1 ml) and 10% palladized carbon (100 mg) refluxed for two hours under a nitrogen atmosphere. The hot reaction mixture was filtrated over a column of Celite; the column was flushed with 40 ml of ethanol. The solvent was removed at reduced pressure. There was obtained a white foam which was purified by flash chromatography (SiO_2 / diethyl ether). The yield of the pure compound amounted to 300 mg (42% overall).

NMR (CD_3COCD_3): 1.32 (d,3H), 3.73 (s,1H), 6.2 (m,2H).

5. References

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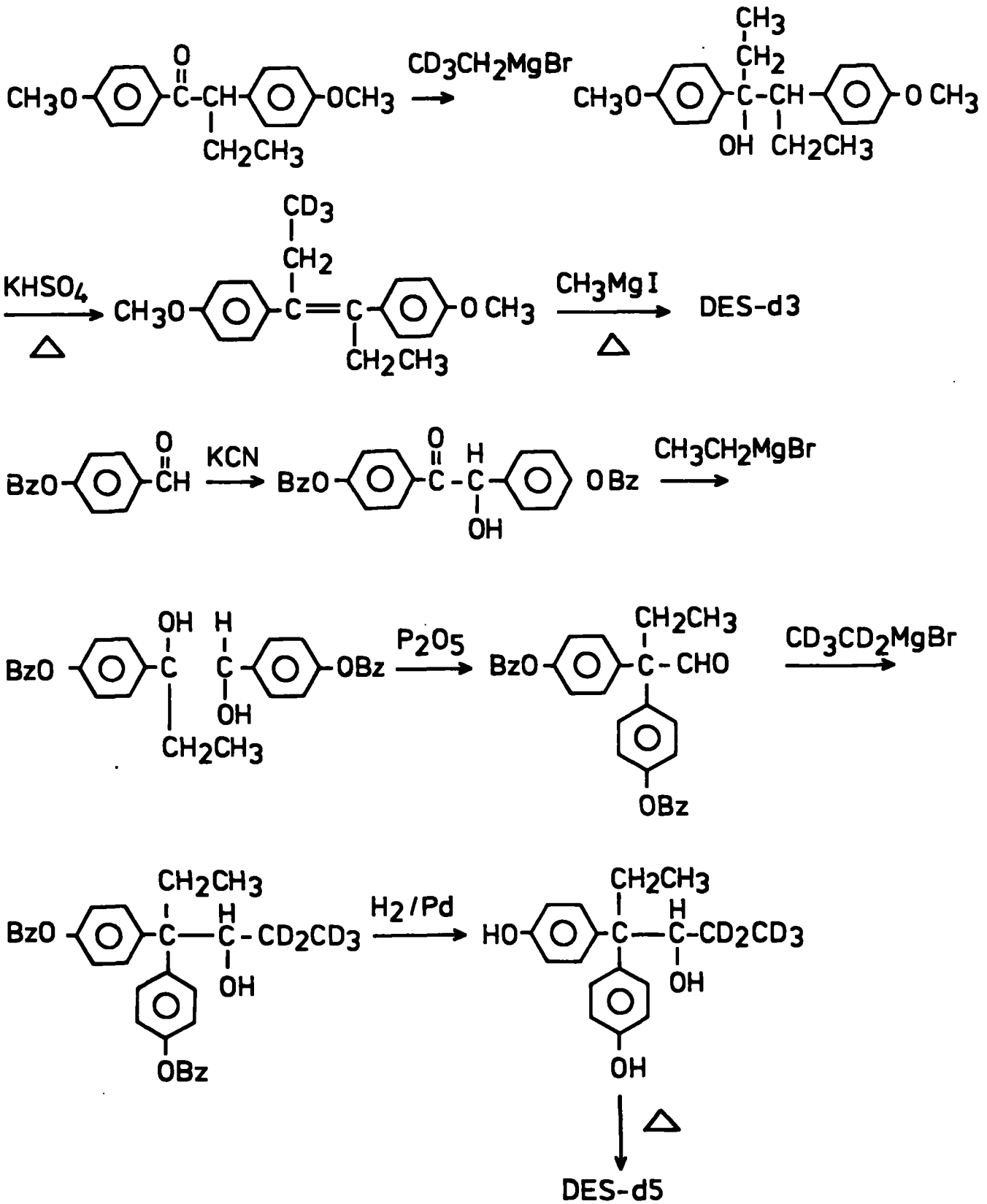
Appendix 1


CH3O
O
C-CH
OCH3
CH2CH3
CD3CH2MgBr
CH3
CH2
OH
CH2CH3
KHSO4
CD3
CH2
CH3MgI
DES-d3
BzO
KCN
OBz
CH3CH2MgBr
P2O5
CD3CD2MgBr
CHO
CD2CD3
H2/Pd
HO
DES-d5

Appendix 2:

Used trivial names with "Chemical Abstracts" names [C.A.S. nr.]

diethylstilbestrol (DES) : Phenol,4,4´-(1,2-diethyl-1,2-ethylenediyl)bis (E)
[56-53-1]

hexestrol (HEX) : Phenol,4,4´-(1,2-diethyl-1,2-ethanediyl)bis-(R*,S*)
[84-16-2]

dienestrol (DE) : Phenol,4,4´-(1,2-diethylidene-1,2-ethanediyl)bis
[84-17-3]

methyltestosterone : Androst-4-en-3-one, 17hydroxy-17-methyl (17β)
[58-18-4]

zeranol (Z) : 1H-2-Benzoxacyclotetradecin-1-one,3,4,5,6,7,8,9,10,11,12-decahydro-7,14,16-trihydroxy-3-methyl, [3S-(3R*,7S*)]
[26538-44-3]

trenbolone (TB) : Estra-4,9,11-trien-3-one, 17hydroxy-,(17β)
[10161-33-8]

nortestosterone (NT) : Estr-4-en-3-one, 17-hydroxy-,(17β)
[434-22-0]

medroxyprogesterone (MP) : Pregn-4-ene-3,20-dione,17-hydroxy-6-methyl-(6α)
[520-85-4]

p-hydroxypropiophenone : 1-Propanone,1-(4-hydroxyphenyl)
[70-70-2]

p-methoxypropiophenone : 1-Propanone,1-(4-methoxyphenyl)
[121-97-1]

p-anysoylchloride : Benzoylchloride,4-methoxy
[100-07-2]

dehydroisoandrosterone : Androst-5-en-17-one,3-hydroxy-(3β)
[53-43-0]

trendione : Estra-4,9,11-triene-3,17-dione
[4642-95-9]

17-hydroxyprogesterone : Pregn-4-ene-3,20-dione,17-hydroxy
[68-96-2]

zearalenone : 1H-2-Benzoxacyclotetradecin-1,7(8H)-dione,3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-,[S-(E)]
[17924-92-4]